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PLANT PROTECTION BULLETIN

A PUBLICATION OF THE WORLD REPORTING SERVICE ON PLANT DISEASES AND PESTS

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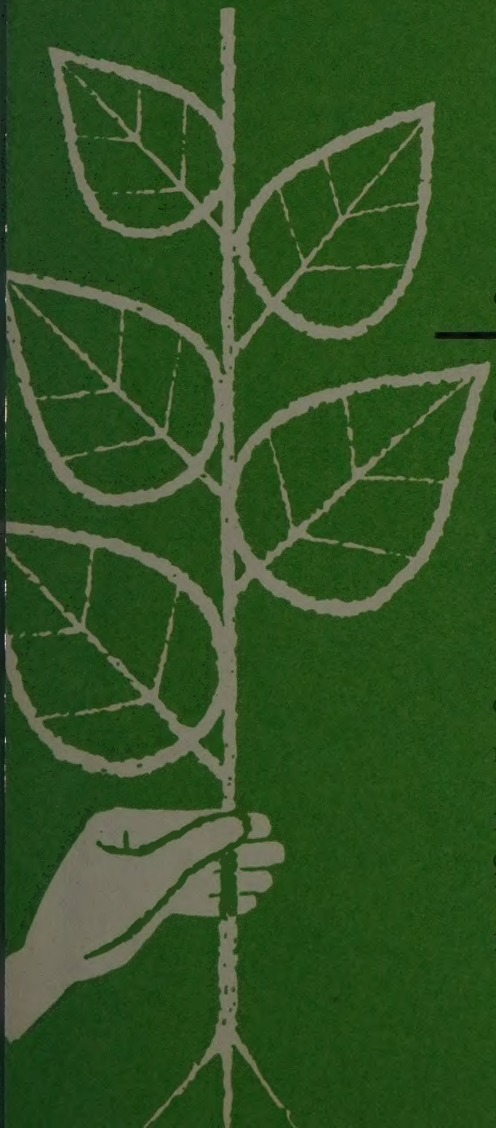

CONTENTS

Collaborative Pesticides Analytical Committee of Europe

R. de B. Ashworth 17

Recommended analytical methods for pesticides

1. Mercury in formulated or technical products

Collaborative Pesticides Analytical Committee 19

Outbreaks and new records 29

Italy

Plant quarantine announcements 30

Canada, Federal Republic of Germany, United Arab Republic (Syrian Region)

News and notes 32

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FAO PLANT PROTECTION BULLETIN

A PUBLICATION OF THE WORLD REPORTING SERVICE ON PLANT DISEASES AND PESTS

Collaborative Pesticides Analytical Committee of Europe

R. de B. Ashworth, Plant Pathology Laboratory, Harpenden, Herts, England

At the ninth session of the European Commission on Agriculture of FAO held in Rome in June 1957, a resolution was passed recommending to Member Governments that studies should be made of the methods of analysis of crop protection products. As a consequence of this recommendation and discussions on the need for internationally agreed methods for the evaluation of pesticides, which took place in a small group of scientists at the Fourth International Congress of Crop Protection in Hamburg in September 1957, the Collaborative Pesticides Analytical Committee (CPAC) was established.

The Third International Congress of Crop Protection which met in Paris in 1952 passed a resolution which resulted, at a meeting at Montdorf-les-Bains in Luxembourg, in the appointment of a committee with somewhat similar interests to CPAC, under the chairmanship of Professor J.A.A. Ketelaar of the Netherlands and with Dr. J.L.H.A. Prat of France as secretary. This group had done much valuable preliminary work but was now replaced, as a consequence of a resolution of the Fourth Congress, by CPAC, which comprises the representatives of a group of European countries. After the preliminary discussions in Hamburg, a second meeting of CPAC was held in England, when the basic aims and procedure were discussed and agreed. Methods for residue analysis are expressly excluded, and it is not proposed at present to consider specifications for pesticides.

In all, four meetings of CPAC have now been held with Professor Ketelaar as chairman and the writer as secretary. Recently, Professor Ketelaar found it necessary to resign, on taking up an industrial appointment and, at the conclusion of the meeting held in Paris in June 1960, Dr. Prat was unanimously elected to replace him for the next four years.

The terms of reference of CPAC are :

" In the first instance, to organize collaborative work on the preparation of agreed analytical methods for materials and their formulations used in crop protection as insecticides, fungicides, herbicides, rodenticides, attractants and repellents. The analytical methods shall include those necessary for the determination of the chemical composition and the physical properties of the materials and their formulations. "

While not proposed at present, it will be appreciated that agreement on methods of analysis is an important step forward should the need arise for agreement on specifications for pesticides.

The countries which are at present taking part in this work are Belgium (Dr. J. Henriet, Gembloux); France (Dr. J.L.H.A. Prat, I.R.C.A.); Germany (Dr. H. Zeumer, Braunschweig) ; Netherlands (Dr. L. Westenberg, Wageningen) ; Scandinavia (Dr. O.A. Martins, Springforbi) ; Switzerland (Dr. C. Zäch, Wädenswil), and the United Kingdom (Dr. R. de B. Ashworth, Harpenden). It will be seen that each country is represented by a leading official concerned with

the chemical evaluation of pesticides. A notable feature of CPAC has been the unselfish team work shown by all members.

Moreover, while it would not be practicable for the pesticide industry of each country to be represented on the committee, yet CPAC members do in fact receive very considerable assistance and guidance from the industry of their country. Thus, in the United Kingdom the Ministry of Agriculture, Fisheries and Food has set up a Pesticides Analysis Advisory Committee (PAC) under the chairmanship of the writer, with one of its aims to provide guidance on CPAC matters. Of the twelve members of this committee, half are leading analysts from industry, while the other six are distinguished official workers. Scandinavia has also set up a committee to cover her special needs, while other member countries have formed rather similar agencies.

The procedure followed by CPAC is briefly as follows. Chemicals for which agreed methods of analysis are desired are allocated to member countries on the basis of origin of the chemical, where it is manufactured, and the availability of facilities for the necessary analytical investigations. Once a chemical has been allocated, it becomes the responsibility of the country concerned to investigate, by collaboration between a number of laboratories, possible methods of analysis. When a suitable method has been selected, it is submitted together with a report giving the results of collaborative work carried out to CPAC, for acceptance as a Draft CPAC Method. Provided no objections are received within twelve months from member countries, the method may then be adopted as an agreed CPAC Method. If objections are raised, the method may still be published as a Draft Method, accompanied by a statement of the objections, or another method may be substituted. Where methods are considered to be of exceptional importance, CPAC itself may undertake the organization of collaborative work.

In accordance with its desire to promote any useful international activity in the field of crop protection, FAO has generously offered space in the *FAO Plant Protection Bulletin* for the publication of those methods accepted by CPAC. So far about twenty methods have been accepted as CPAC Methods and a number of others as Draft CPAC Methods. These will be published from time to time in this Bulletin as space permits. They can, with due acknowledgement to CPAC, be reissued in the technical journals of any individual country. Other methods are in course of preparation and every endeavor will be made to replace older methods by new and better ones as soon as they are recognized as such. It is very much hoped that comments and suggestions for improvement will be received from those who use these CPAC Methods.

Recommended Analytical Methods for Pesticides

1. Mercury in formulated or technical products [1]

Collaborative Pesticides Analytical Committee,
c/o Plant Pathology Laboratory, Harpenden, Herts, England

Introduction

The analysis of formulated mercury products for mercury presents considerable difficulty because of the presence of fillers, lead arsenate, solvents, surface active agents, coloring matter, BHC and other halogenated compounds. Methods of analysis given in the literature are based on the decomposition of the compound with strong inorganic acids, bromine and acetic acid, zinc and sulphuric acid, or heating with basic substances such as lime. The mercury may then be weighed as such or determined as ionic mercury in a variety of ways. However, in general, gravimetric methods have been found unreliable by the members of CPAC for the determination of mercury in pesticides. By selection of a method of decomposition, combined with a suitable method of determination, numerous methods of analysis become theoretically possible.

The volatility and stability of organo-mercury compounds vary considerably and so influence the choice of a method of analysis. The methyl and ethyl derivatives are very stable to many reagents and are somewhat volatile ; the phenyl are less stable, while alkoxymercury compounds are relatively easily decomposed. Both phenyl and alkoxyethyl derivatives are comparatively non-volatile.

Accordingly, until a universal method has been developed, it is important to know something of the composition of the sample before undertaking an analysis. Table 1 gives three basic procedures (HG1, HG2 or HG3), together with minor modifications for particular formulations, and will be found helpful in making a choice.

Table 1. Selection of Method of Analysis*

Mercury in the presence of	Basic procedure		
	HG 1	HG 2	HG 3
Aldrin	—	**2 (iii)**	3 (ii)
Aqueous solutions	—	**2 (i)**[a]	3 (i)
BHC	—	**2 (iii)**	3 (ii)
Calcium carbonate	—	**2 (iii)**	3 (iii)
Calcium carbonate plus BHC, aldrin or dieldrin	—	**2 (iii)**	3 (iv)
Captan	—	**2 (iv)**	—
Chlorides	—	**2 (i)**	3 (i)
Copper	**1**	No	No
Dieldrin	—	**2 (iii)**	3 (ii)
Dyestuffs	—	**2 (i)**	3 (i)
Heptachlor	—	**2 (iii) B**	—
High fat content	—	No	3 (i)
Iron blue pigments (e.g., Prussian blue)	—	**2 (i)**	No
Lead arsenate	1	**2 (i)**	No
Organic solvents[b]	—	**2 (ii)**	3 (i)
Organic solvents[c]	—	**2 (iii)**	3 (i)
Organic solvents[d]	—	No	3 (i)
Sulphite lye[e]	—	**2 (i) or 2 (ii)**	3 (i)

* Preferred method in bold type.

[a] In the case of samples containing 1.0 percent or less of mercury, use method HG 3 (i).

[b] Where the solvent is readily and safely decomposed by sulphuric acid, e.g., ethylene glycol.

[c] Where the solvent reacts dangerously with sulphuric acid or nitric acid.

[d] Where the solvent has with it large amounts of fatty material.

[e] If large amounts of sulphite lye are present, method HG 2 (ii) should be used.

[1] These methods were accepted in June 1960 by the Collaborative Pesticides Analytical Committee (CPAC), which consists of scientists from seven European countries having a substantial chemical industry. The original method is in English.

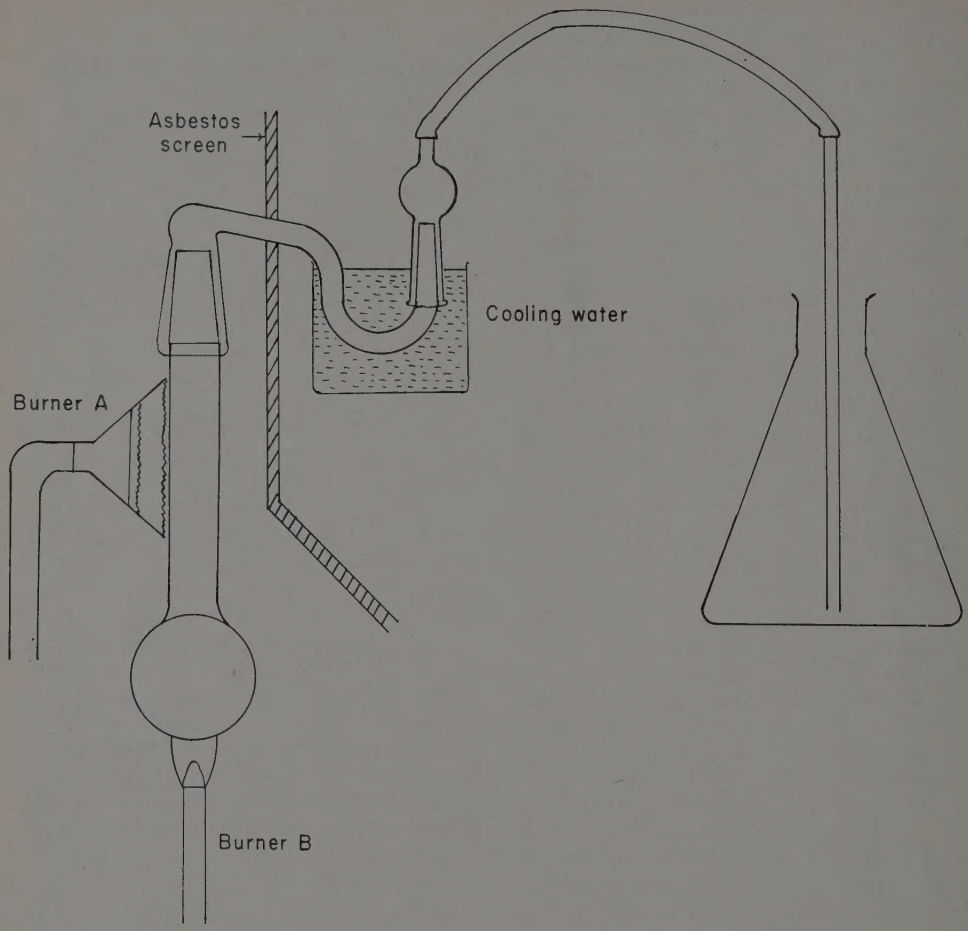

Figure 1. Distillation apparatus

HG 1 Distillation Method [2]

Principle. The sample is decomposed by heating with calcium oxide and sucrose. The liberated mercury is dissolved in nitric acid and then titrated with ammonium thiocyanate.

Scope. This method is the preferred method for the determination of mercury in the presence of copper, and has also given satisfactory results for mercury in the presence of lead arsenate.

Apparatus. The complete apparatus is shown in Figure 1, consisting of :

25 ml. round-bottom fused silica flask of bulb capacity approximately 25 ml. ; the neck is 1.2 cm. in diameter and 11 cm. long (including the B14 cone, which is 2.8 cm. long) and glass stopper to fit (Figure 2)

Receiver - borosilicate glass (dimensions as shown in Figure 3)

[2] This method is based on that given in Brookes, H.E. and L.E. Solomon. The determination of mercury by distillation from its compounds and preparations. *Analyst* 84 (1004) : 622-629. 1959.

Bunsen burner A - made from a piece of brass tubing 10 cm. × 1.3 cm. (4 inches × ½ inch). For convenience in heating the neck of the flask, fit a piece of 1.3 cm. (½ inch) diameter copper tubing bent in a right angle, and attach a 4.5 cm. flame spreader by means of Emerto gas pipe fittings

Bunsen burner B - made from a piece of 9 cm. × 1 cm. ($3^1/_2$ inches × $^7/_{16}$ inch) brass tubing

Asbestos screen - a suitably shaped piece of asbestos to shield the receiver and beaker of water from the heat

150 ml. beaker - tall form

Steel wool (Note 1)

Glass wool - wash with concentrated nitric acid, distilled water, and then dry

Silicone grease

Iron wire

Weighing bottle

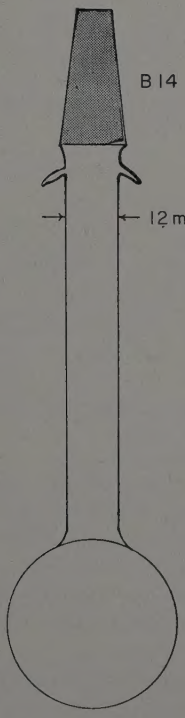

Figure 2. Fused-silica flask

Filter funnel

500 ml. conical flask

500 ml. conical flask - wide-mouthed

25 ml. measuring cylinder

Steam bath

25 ml. burette

Reagents

Carbon tetrachloride

Sucrose

Calcium oxide, reagent grade, particle size 20-40 mesh

Iron filings, made from electrolytic iron (20-40 mesh)

Zinc wool

Ammonium thiocyanate, 0.1N solution

Ammonium ferric sulphate (Note 2)

Potassium permanganate, 5 percent w/v solution

Ferrous sulphate

Acetone

Nitric acid

Aerosil 2491 (Note 3)

Method. Take a representative sample of at least 100 gm. Weight accurately sufficient of the sample (*w* gm.) to contain at least 50 mg. of mercury (Note 4). Mix with sucrose (Note 5) (2 gm.) and transfer to the flask (Note 6) by means of the funnel.

Fill the bulb of the flask up to the neck with iron filings, then fill the neck up to the bottom of the ground glass cone with a mixture of equal parts by volume of iron filings and calcium oxide. Fit the cone with a tight plug of steel wool.

Plug the B10 joint of the receiver with zinc wool and prepare the glass wool trap by stuffing the bulbous portion with glass wool retained by a zinc wool plug. Attach the receiver to the trap by the B10 joint and fix by a spring. Join a length of narrow rubber tubing to the other end of the trap with a small glass bubbler on the end for passing evolved gases into carbon tetrachloride contained in a conical flask.

Lubricate the B14 joint of the flask with silicone grease, attach to the receiver and secure by tying iron wire round the lugs provided. Clamp the flask vertically with the U-bend of

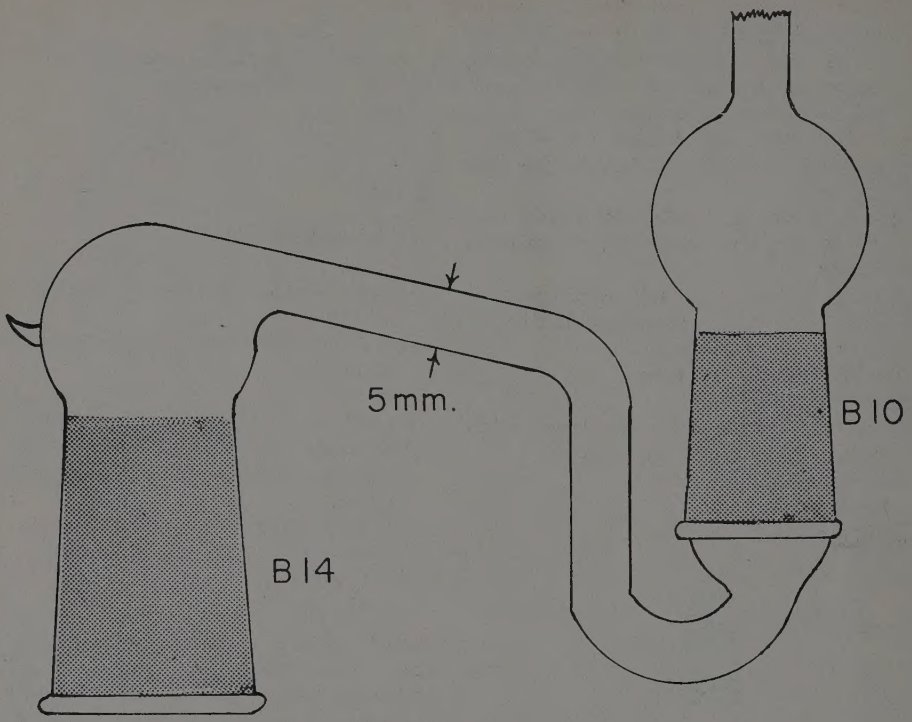

Figure 3. Borosilicate-glass receiver

the receiver immersed in cold water in the beaker. Introduce the asbestos screen between the cooled portion of the receiver and the flask.

Heat the neck of the flask to a dull red for five minutes with burner A (Note 7) and, while continuing to heat, heat the bulb of the flask with burner B, using an extremely small flame, so that the evolution of gas begins in five to ten minutes. Adjust burner B so that there is a steady evolution of gas over about 15 minutes. When the production of gas is considerably reduced, heat for another two minutes with the full heat of burner B (Note 8). A good indication of the rate of evolution of the gas can be obtained from the position of the "ring" of condensing mercury globules in the receiver, which should pass about 1 inch beyond the upper bend of the receiver.

At the end of the heating period, turn off both burners and cut the iron wire. Detach the receiver from the flask and trap, allow the receiver to cool for about 15 minutes, wash free from tarry matter by pouring acetone through the B14 socket, and then wash acetone-free with water.

Put the receiver in the wide-mouthed conical flask, add water (15 ml.), and then nitric acid (15 ml.), slowly at first, until most of the zinc dissolves. Warm on a steam bath to dissolve the mercury and then boil the solution to remove nitrous fumes. Add the potassium permanganate solution to dropwise until a permanent pink color is obtained (Note 9), cool, and decolorize by adding ferrous sulphate. Dilute with water to about 120 ml., cool to below 15° C., and titrate with the ammonium thiocyanate

solution, using the ammonium ferric sulphate solution as indicator (t ml.).

$$\text{Percentage mercury w/w} = \frac{1.003\,t}{w}$$

NOTE 1. Grade 0 Supreme, obtained from the Brillo Manufacturing Company of Great Britain, Ltd., North Circular Road, London, N.W. 10, is suitable.

NOTE 2. Dissolve ammonium ferric sulphate (15 gm.) in distilled water (100 ml.), and add sufficient concentrated nitric acid (nitrous acid-free) to discharge the brown color. The color of the resultant solution should be a pale yellow color.

NOTE 3. Aerosil is obtainable from Bush, Beach, Segner and Bailey Ltd., 44, Brazenose Street, Manchester, 2.

NOTE 4. Where a liquid is to be analyzed, pipette, without allowing the liquid to touch the sides, x cc's of sample into the tarred stoppered reaction flask containing 0.2 gm. of Aerosil. Replace stopper and weigh flask. Rotate flask to mix the contents until a dry powder is obtained.

$$\text{Percentage mercury w/v} = \frac{1.003\,t}{x}$$

NOTE 5. Sucrose must be added to preparations that contain inorganic mercury compounds or are difficult to decompose. When ignited with mercurous chloride the sucrose produces enough gas to clear the flask of mercury in about 20 minutes at a steady rate of evolution. Volatile mercury compounds (e.g., methyl mercury chloride) also require sucrose.

NOTE 6. A small amount of calcium oxide mixed with the powder helps it to flow more freely.

NOTE 7. A piece of wire gauze round the standard joint protects it from the direct heat of the flame.

NOTE 8. Because of great dissimilarity in mercury-containing preparations, it is not possible to state exact heating periods for the whole range of preparations; however, a total heating time of 15 to 20 minutes is generally ample.

NOTE 9. If more than one or two drops of potassium permanganate solution are required, it is usually an indication that lower oxides of nitrogen are still present.

HG 2 Sulphuric Acid-Potassium Nitrate Oxidation Method [3]

Principle. The sample is refluxed with concentrated sulphuric acid and potassium nitrate and the mercury determined volumetrically with thiocyanate.

Where large amounts of calcium carbonate or highly chlorinated compounds (e.g., BHC) are present, the mercury is isolated as a sulphide before conversion to the ionic form with strong acid. Other minor modifications are necessary in special cases.

Reagents

Sulphuric acid, concentrated
Potassium nitrate, 5 gm. tablets or cast sticks (Note 1)
Potassium permanganate, saturated solution in water
Ammonium ferric sulphate (Note 2 under HG 1)
Potassium or ammonium thiocyanate, 0.1N solution
Potassium or ammonium thiocyanate, 0.05N solution
Hydrochloric acid, 1 + 1 solution
Ethanol or industrial methylated spirit
Whatman No. 5 filter paper (or equivalent), 7 cm. diameter
Asbestos for Gooch crucibles
Hydrochlorid acid, 2N solution
Sodium hydroxide, approximately 5N solution
Hydrogen sulphide
Ferrous sulphate, approximately 0.2M solution

Apparatus

Weighing scoop for solids *or*
Weighing bottle for liquids
2 100 ml. measuring cylinders
10 ml. measuring cylinders
500 ml. conical flask, B34 socket
750 ml. conical flask, B34 socket
Air condenser, 150 cm. long, 2.5 cm. diameter, with B34 cone (Note 2). There should be no restriction below the ground joint (Note 3)
Liebig condenser, minimum length 46 cm., with B34 joints
Buchner funnel, 8.7 cm. diameter to take 7 cm. filter papers or 6.5 cm. diameter with sintered glass plate porosity No. 2
1,000 ml. filter flask
Sintered glass crucible, porosity No. 2 or 3 (i.e., in the range 20 to 50 microns pore

[3] The early work of the Mercury Committee is described in Chambers, V.H., F.R. Cropper and H. Crossley. The analysis of organic mercurials. *Jour. Sci. Food. Agr.* 7: 17. 1956.

size), small enough to pass through a B34 joint (Note 4)

Sampling. Take a representative sample of not less than 100 gm.

SECTION HG 2(i)

Scope. For solids free from highly chlorinated organic compounds (e.g., BHC), sulphite lye, or calcium carbonate and liquids, except those containing a high proportion of organic matter, in which case see HG 2 (ii).

Method. WARNING. — It is dangerous to use this wet oxidation method in the presence of any organic material which reacts violently with sulphuric acid or nitric acid.

Weigh accurately sufficient of the sample (*w* gm.), to contain between 0.15 and 0.25 gm. of mercury (Note 5), and transfer to the dry 750 ml. conical flask.

Add potassium nitrate (2 × 5 gm. tablets). Measure 50 ml. concentrated sulphuric acid and pour the first few milliliters into the flask over the ground joint in order to rinse in any of the sample which may be adhering. Except where a carbonate filler is present, pour the remainder of the acid direct into the flask and attach the air condenser. If a carbonate filler (other than calcium carbonate) is present, attach the condenser first and pour the remainder of the acid down the condenser. Transfer the flask to the source of heat and bring the acid mixture to the boil (Note 13).

Continue as under Section HG 2 (v).

SECTION HG 2 (ii)

Scope. For solids containing a high proportion of organic matter (other than highly chlorinated compounds) or a high sulphite lye content, and for liquids containing certain organic solvents, such as ethylene glycol, which may be safely oxidized with nitric or sulphuric acid. (For samples containing a high fat content Method HG 3 (i) should be used.)

Method. Weigh accurately sufficient of the sample (*w* gm.), to contain between 0.15 and 0.25 gm. of mercury (Note 5), and transfer to the dry 750 ml. conical flask.

Measure 50 ml. concentrated sulphuric acid and pour the first few milliliters over the ground joint in order to rinse in any of the sample which may be adhering. Except where a carbonate filler is present, pour the remainder of the acid direct into the flask and attach the air condenser. If a carbonate filler (other than calcium carbonate) is present, attach the condenser first and pour the remainder of the acid down the condenser. Transfer the flask to the source of heat and bring the acid mixture to the boil. Reflux for about 15 minutes in order to char the organic matter thoroughly, remove from source of heat, then cautiously add through the condenser small pieces of potassium nitrate (2 × 5 gm. tablets) (Note 6).

Continue as under Section HG 2 (v).

SECTION HG 2 (iii)

Scope. For solids or liquids containing highly chlorinated compounds (e.g., BHC) and/or calcium carbonate (Note 7).

Method. Weigh accurately sufficient of the sample (*w* gm.), to contain between 0.15 and 0.25 gm. of mercury, and in no case exceeding about 10 gm. of sample, and transfer to the dry 500 ml. conical flask. Attach the water-cooled reflux condenser and pour down it dilute hydrochloric acid (50 ml., 1 + 1 H_2O). Boil gently for one hour with occasional shaking.

Cool for a moment (i.e., until refluxing ceases), add cold water (50 ml.) and shake the flask well in order to prevent any chlorinated compound present from coalescing into large lumps. Remove the condenser and complete the cooling of the flask by immersing it in cold water. Filter the flask contents with suction through a Buchner funnel fitted with a No. 1 or similar filter paper on which is placed a thin layer of Gooch asbestos, or through a sintered glass plate and Gooch asbestos. Wash the flask and the filter thoroughly with several small portions of cold water, taking care not to allow the filter to suck completely dry until the washings are complete.

Continue with either Sub-Section HG 2 (iii) A or B below (Note 8).

Sub-Section HG 2 (iii) A. Transfer the filtrate (about 400 ml.) to the 750 ml. conical flask

and add 5N sodium hydroxide solution until only a faint purple color shows on Congo red paper, add 2N hydrochloric acid (2 ml. for each 100 ml. of solution), warm the solution to 40° to 50° C., and bubble in hydrogen sulphide for at least 15 minutes.

Allow the sulphide precipitate to settle, then filter through the small sintered glass crucible fitted with an asbestos pad. Wash the precipitate and flask with warm water until the washings are free from chloride (Note 9). Suck dry, then transfer the crucible and its contents back to the flask in which the precipitation was carried out. Add potassium nitrate (3 × 5 gm. tablets) and concentrated sulphuric acid (50 ml.). Attach the air-cooled reflux condenser, bring the acid mixture to the boil, and digest for not less than one hour.

Continue as under Section HG 2 (v).

Sub-Section HG 2 (iii) B. Transfer the filtrate to the 500 ml. conical flask (Note 10) fitted with a water-cooled reflux condenser. Heat under reflux until the mixture is boiling gently, add down the condenser zinc wool (1 gm.) rolled into a ball, continue boiling for 15 minutes, repeat with a further two lots of zinc wool (3 × 1 gm. in all). Remove the source of heat, rinse down the condenser with a little acetic acid, allow to cool until the flask can be conveniently handled and detach the flask.

Fit the Buchner funnel with two 5.5 cm. filter papers and cover them with a layer of Gooch asbestos (see Note 1 under HG 3). Decant the hot contents of the flask onto the filter, taking care to retain the zinc (which may have partly disintegrated) in the flask as far as possible.

Rinse the flask with water and pass the washings through the filter. Wash the residue in the flask and the filter until the washings are free from halides, as shown by testing with silver nitrate.

Continue as under Section HG 3 (v).

SECTION HG 2 (iv)

Scope. For samples containing about 40 percent captan and about 1.5 percent mercury.

Method. Weigh accurately about 5 gm. of the sample (*w* gm.), and transfer to the dry 750 ml. conical flask. Measure 50 ml. concentrated sulphuric acid and pour the first few ml. over the ground joint in order to rinse in any of the sample which may be adhering. Except where a carbonate filler is present, pour the remainder of the acid direct into the flask and attach the air condenser. If a carbonate filler (other than calcium carbonate) is present, attach the condenser first and pour the remainder of the acid down the condenser.

Heat and bring the acid mixture to the boil. Reflux for about two and a half to three hours in order to char the organic matter thoroughly, remove from the source of heat, then cautiously add, through the condenser, small pieces of potassium nitrate (2 × 5 tablets). Reflux for one hour, then add a further 10 gm. of potassium nitrate as before.

Continue as under Section HG 2 (v), except that 0.05N thiocyanate solution should be used.

$$\text{Percentage mercury w/w} = \frac{0.5015\,t}{w}$$

where t = ml. of 0.05N thiocyanate solution

SECTION HG 2 (v)

Continued from end of Sections HG 2 (i, ii, iiiA, or iv).

Continue refluxing for 30 minutes for technical organo-mercury compounds and formulated products containing an acid-soluble filler. Where lead arsenate or an insoluble filler is present, reflux for a minimum of one hour, while in the case of captan, the presence of organo-mercury chlorides reflux for two hours (Note 11).

When the digestion is complete, allow the flask to cool to near room temperature without removing the condenser. Dilute the contents of the flask with water (100 ml.) poured down the condenser in small portions, with swirling. This may be conveniently carried out with the flask resting on the floor, while supporting the condenser at an angle. Add two or three glass beads to the flask, boil until all the lower oxides of nitrogen have been expelled through

the condenser, then cool to room temperature. Rinse the condenser with cold water (10 to 20 ml.) and remove from the flask.

Check the completeness of the oxidation by adding potassium permanganate solution dropwise until a definite pink color persists for five minutes (Note 12). Discharge the permanganate color with a minimum of ferrous sulphate solution, then add ferric alum indicator (5 ml.) and titrate the mercury with 0.1N thiocyanate solution (t ml.).

$$\text{Percentage mercury w/w} = \frac{1.003\,t}{w}$$

In the case of liquids the density d should be determined.

$$\text{Percentage mercury w/v} = \frac{1.003\,t\,d}{w}$$

NOTE 1. Tablets or cast sticks are necessary in order to avoid a too violent initial reaction with the sulphuric acid.

NOTE 2. The air condenser is of sufficient length to give efficient refluxing of the nitric and sulphuric acids. The upper part of the condenser should remain cool during the digestion.

NOTE 3. The absence of constriction below the joint of the condenser ensures that the returned acids run down the sides of the flask. Little or no bumping occurs even if a considerable quantity of insoluble filler is present.

NOTE 4. The crucible should be washed with hot *aqua regia*, rinsed with water, and dried.

NOTE 5. In the case of liquid samples the amount taken should not exceed 10 ml., to avoid diluting the sulphuric acid excessively.

NOTE 6. More than two tablets of potassium nitrate may be necessary, if it is observed that the whole of the nitric acid is reduced and expelled as the lower oxides of nitrogen before the oxidation of the organic matter is complete.

NOTE 7. Pretreatment, as under HG 2 (iii), using twice the amount of hydrochloric acid, is also necessary when calcium carbonate is present, as calcium sulphate, which will be formed by the direct digestion method, occludes mercury strongly, and low results will be obtained.

NOTE 8. In the case of samples containing heptachlor, use Section HG 2 (iii) B only. For other samples either HG 2 (iii) A or B can be used.

NOTE 9. Check the washings with a silver nitrate solution.

NOTE 10. If preferred, the hydrochloric acid digest can be filtered directly into the conical flask.

NOTE 11. Substances containing moderate amounts of chloride such as phenyl mercuric chloride, ethyl mercuric chloride or mercurous chloride (calomel) may be determined by direct digestion as given in HG 2 (i) and (ii), but highly chlorinated compounds, if present, must be removed by the pretreatment given in HG 2 (iii).

NOTE 12. Oxidation of the mercury is complete and no reducing substances should be present after the oxides of nitrogen have been removed. If more than one or two drops of potassium permanganate solution are required, it is usually an indication that the digestion has not been continued for a sufficient length of time, or that lower oxides of nitrogen are still present.

NOTE 13. If the potassium nitrate used is not sufficient to oxidize the sample, use HG 2 (ii), except in the case of captan, then use HG 2 (iv).

HG 3 Zinc-Acetic Acid Method[4]

Principle. The mercury compounds are decomposed with glacial acetic acid and zinc wool. The mercury-zinc amalgam is isolated and decomposed with nitric acid. The mercury is titrated with thiocyanate.

Reagents

Acetic acid, glacial
Acetic acid, solution 1 + 2
Silver nitrate, 0.1N solution approximately
Nitric acid, concentrated
Zinc wool
Potassium permanganate, saturated solution
Ferrous sulphate, approximately 0.2M solution
Ammonium ferric sulphate (see Note 2 under HG 1)
Ammonium or potassium thiocyanate, 0.1N solution

Apparatus

Weighing bottle
250 ml. flask with ground joint
46 cm. water-cooled reflux condenser to fit the flask
10 ml. measuring cylinder
2 100 ml. measuring cylinders
Buchner funnel to take 5.5 cm. filter papers
500 ml. filter flask
Whatman No. 1 filter paper (or equivalent) 5.5 cm. diameter

[4] See footnote 3.

Gooch asbestos
Knife spatula
11 cm. Petri dish
Buchner funnel 6.5 cm. diameter fitted with sintered glass plate, porosity No. 3 (i.e., 20 to 30 micron pore size) *or* Gooch crucible fitted with asbestos pad
25 ml. burette

Sampling. Take a representative sample of not less than 100 gm.

SECTION HG 3 (i)

Scope. For solids or liquids known to be free from highly chlorinated compounds, calcium carbonate, lead arsenate, and iron blue pigments, and for samples containing a high fat content.

Method. Weigh accurately sufficient of the sample (*w* gm.) to contain between 0.15 and 0.25 gm. of mercury, transfer to the 250 ml. flask, add glacial acetic acid (50 ml.), swirl to mix and attach the reflux condenser.

Then continue as under Section HG 3 (v) below.

SECTION HG 3 (ii)

Scope. For solids containing highly chlorinated compounds (e.g., BHC, aldrin, etc.) but no calcium carbonate, lead arsenate or iron blue pigments.

Method. Weigh accurately sufficient of the sample (*w* gm.) to contain between 0.15 and 0.25 gm. of mercury, transfer to the 250 ml. flask, add glacial acetic acid (50 ml.), swirl to mix and attach the reflux condenser. Heat under reflux until the mixture is boiling gently, add down the condenser zinc wool (1 gm.) rolled into a ball, continue boiling for 15 minutes, repeat with a further two lots of zinc wool (3 × 1 gm. in all). Remove the source of heat, rinse down the condenser with a little acetic acid, allow to cool until the flask can be conveniently handled and detach the flask (Note 2).

Fit the Buchner funnel with two 5.5 cm. filter papers and cover them with a layer of Gooch asbestos (Notes 1 and 3). Decant the hot contents of the flask onto the filter, taking care to retain the zinc, which may have partly disintegrated, in the flask as far as possible.

Rinse the flask ten times with hot (about 70° C.) glacial acetic acid (using about 30 ml. each time) and pass the rinsings through the filter. Finally, wash the filter with water (about 20 ml.) to remove most of the acetic acid ; this makes the subsequent handling of the mat easier. Wash the residue in the flask and on the filter with water until the washings are halide-free, i.e., give no precipitate on testing with silver nitrate.

Then continue as under Section HG 3 (v) below.

SECTION HG 3 (iii)

Scope. For solids containing calcium carbonate, but no highly chlorinated compounds, lead arsenate, or iron blue pigments.

Method. Weigh accurately sufficient of the sample (*w* gm.) to contain between 0.15 and 0.25 gm. of mercury, transfer to the 250 ml. flask, add glacial acetic acid (50 ml.), swirl to mix and attach the reflux condenser. Heat under reflux until the mixture is boiling gently, add down the condenser zinc wool (1 gm.) rolled into a ball, continue boiling for 15 minutes, repeat with a further two lots of zinc wool (3 × 1 gm. in all). Remove the source of heat, rinse down the condenser with a little acetic acid, allow to cool until the flask can be conveniently handled and detach the flask.

Fit the Buchner funnel with two 5.5 cm. filter papers and cover them with a layer of Gooch asbestos (Note 1). Add water (20 ml.) to the glacial acetic acid in the flask, avoiding any losses due to effervescence, and then decant the contents of the flask onto the filter, taking care to retain the zinc, which may have partly disintegrated, in the flask as far as possible. Wash the residue in the flask and on the filter until the washings are halide-free (no precipitate with silver nitrate).

Then continue as under Section HG 3 (v) below.

SECTION HG 3 (iv)

Scope. For solids containing chlorinated compounds and calcium carbonate, but no lead arsenate or iron blue pigments.

Method. Weigh accurately sufficient of the sample (*w* gm.) to contain between 0.15 and 0.25 gm. of mercury, transfer to the 250 ml. flask, add glacial acetic acid (50 ml.), swirl to mix and attach the reflux condenser. Heat under reflux until the mixture is boiling gently, add down the condenser zinc wool (1 gm.) rolled into a ball, continue boiling for 15 minutes, repeat with a further two lots of zinc wool (3 × 1 gm. in all). Remove the source of heat, rinse down the condenser with a little acetic acid, allow to cool until the flask can be conveniently handled and detach the flask (Note 2).

Fit the Buchner funnel with two 5.5 cm. filter papers and cover them with a layer of Gooch asbestos (Notes 1 and 3). Decant the hot contents of the flask onto the filter, taking care to retain the zinc (which may have partly disintegrated) in the flask as far as possible.

Rinse the flask ten times with hot (about 70°C.) glacial acetic acid (using about 30 ml. each time) and pass the rinsings through the filter. Add dilute acetic acid to the contents of the flask to dissolve any calcium carbonate, avoiding any losses through effervescence, and pass the rinsings through the filter. Wash the filter with dilute acetic acid until the rinsings are free from halides, as shown by testing with silver nitrate.

Then continue as under Section HG 3 (v) below.

SECTION HG 3 (v)

(*Continuation of Section HG 3 (i) to (iv) above.*)

Remove the residue from the Buchner funnel with a knife spatula and transfer it carefully to a Petri dish. Detach the bottom filter paper from the mat, roll the mat into a cylinder or cut the mat into slices and return it to the flask containing the zinc. Use the bottom filter paper to wipe round the Buchner funnel and the Petri dish in order to remove the last traces of the mat, and add this paper to the flask.

Reassemble the flask and condenser and add cautiously, a few milliliters at a time, concentrated nitric acid (20 ml.). When the vigorous reaction has subsided, gently heat the flask until no more nitrous fumes are evolved, meanwhile swirling the contents so that no part of the residue remains unattacked by the nitric acid. Add water down the condenser (about 20 ml.) and boil the solution until free from fumes (about five minutes). Cool slightly, rinse down the condenser with a little water and detach the flask.

Filter where necessary (Note 1) through the Buchner funnel fitted with the sintered glass plate (or through the Gooch crucible fitted with an asbestos pad) and wash three times with small volumes of water, keeping the total volume of the filtrate below 150 ml. Add saturated potassium permanganate solution dropwise to the flask until the solution is a definite pink color which persists for five minutes. Destroy the excess permanganate with the minimum of ferrous sulphate solution. Add ferric alum indicator solution (5 ml.), cool the solution to laboratory temperature (or slightly below) if necessary, and titrate with 0.1N thiocyanate solution (*t* ml.) to a permanent reddish-brown color.

$$\text{Percentage mercury w/w} = \frac{1.01\,t}{w} \qquad \text{(Note 4)}$$

In the case of liquids, determine the density (*d*).

$$\text{Percentage mercury w/v} = \frac{1.01\,d\,t}{w} \qquad \text{(Note 4)}$$

NOTE 1. If there is little or no solid material in suspension, other than the zinc, a 34 mm. Gooch crucible with asbestos mat is preferable to the Buchner funnel. If a Gooch is used, the subsequent filtration in the last paragraph of the method may be omitted.

NOTE 2. If, in a series of experiments, a flask has been cooled too much, it should be reheated before filtering the contents.

NOTE 3. In the case of products containing chlorinated compounds (e.g., BHC), wash the filter mat with several small portions of glacial acetic acid to remove the water trapped in it.

NOTE 4. The factor 1.01 includes a small correction (0.7 percent) to allow for the slightly low results by this method.

OUTBREAKS AND NEW RECORDS

ITALY

R. Ciferri, Cryptogamic Laboratory, University of Pavia

Serious outbreak of blue mold of tobacco

In July 1960, blue mold of tobacco (*Peronospora tabacina* Adam) occurred almost simultaneously in three provinces in northern Italy, Pavia (Lombardy), Trento (Trentino) and Padova (Veneto). Subtropical tobacco grown under shade-cloth and varieties Nostrano del Brenta and Burley grown in the field without protection were infected.

By mid-September the disease was present in 14 provinces of Lombardy, Trentino and Veneto, all situated in the Po Valley north of the River Po. The infested area extended from the River Po to the subalpine region of the Provinces of Trento and Udine, and from the Provinces of Asti and Alexandria to the subalpine and Adriatic belt of the Provinces of Udine, Padova and Mantova.

The losses caused by blue mold were extremely severe. It has been estimated that in Lombardy alone 300 hectares, the equivalent of 20 percent of the area planted to tobacco, were destroyed by the disease. All tobacco grown under shade suffered from a complete loss. Serious but variable losses occurred in the Burley variety, whereas Nostrano del Brenta and other local varieties appeared to be less susceptible.

The causal fungus found in Italy is not morphologically distinguishable in its sexual or conidial stage from the Australian or American strains.

According to information received by the writer, this disease occurred in 1958 in four different places in southeastern England. In 1959 it was found in the Netherlands and in 1960 in Belgium, France, Germany and Austria, as well as in the Netherlands.

PLANT QUARANTINE ANNOUNCEMENTS

CANADA

The Destructive Insect and Pest Regulations of 22 December 1954 (see *FAO Plant Prot. Bull.* 3 : 110. 1955) was amended by Order in Council P. C. 1960-148 of 11 February 1960, published in the *Canada Gazette* (Part II) Vol. 94, No. 4 of 24 February 1960. The major changes concern the importation of nursery stock and of soil with respect to the golden nematode and potato wart.

Nursery stock

The phytosanitary certificate accompanying shipment of nursery stock from countries other than the United States (except Hawaii) where golden nematode or potato wart is known to occur should include the following declaration : " The nursey stock included in this shipment was grown in soil in which the potato wart disease, *Synchytrium endobioticum* (Schilb.) (Perc.) has not been recorded, and in which, based on official soil surveys and other precautionary practices, the golden nematode, *Heterodera rostochiensis* (Wr.), is not known to occur. "

Nursery stock may be imported through the following ports : St. John's (Newfoundland), Corner Brook, Halifax, Saint John (New Brunswick), Quebec, Monteal, Ottawa, Toronto, Niagara Falls, London, Windsor, Winnipeg, Estevan, Lethbridge, Edmonton, Vancouver, Victoria.

Plants with soil

Importation of plants and parts thereof with sand, soil or earth around the roots and plant packing material containing sand, soil or earth is prohibited, except those from the United States (excluding Hawaii) and from Belgium, Denmark, England and Wales, the Netherlands and the Federal Republic of Germany (Provinces of Schleswig-Holstein and Oldenburg). If imports originate from the European countries mentioned above, the plants must have been grown in nurseries in which all sanitary requirements with respect to serious plant pests and diseases, including golden nematode and potato wart, have been carried out, provided that records in this connection and complete description of the nursery are maintained by an official certifying authority. Certificates of freedom from pests and diseases in general and golden nematode and potato wart in particular should be issued only for plants grown on land in which golden nematode and potato wart are not known to occur, and in which the growing of potatoes, tomatoes and other hosts of golden nematode has not been permitted for at least three years prior to the year of export certification, or for a shorter time in case of bulbs substantially free from soil.

Lily bulbs packed in clean ocean sand or coral sand without surface soil, or in a subsoil which has been sieved, dried and stored in a manner to prevent contamination is not subject to the prohibition mentioned above. A declaration that this requirement has been met should be included in the certificate.

The provision in the 1954 Regulations restricting the importation of plants with soil from Asia is revoked.

Plants without soil

Plants and parts thereof, free from sand, soil or earth, from countries where the golden nematode or potato wart occurs are prohibited, unless the special declaration mentioned under " Nursery stock " is included in the certificate.

The declaration is not required with respect to imports from the United States (except Hawaii), also scions and budsticks, and plants grown in media other than sand, soil or earth. If coming from countries other than the United States (except Hawaii) where golden nematode and potato wart are not known to occur, they should be covered by a declaration to that effect in the certificate.

Soil, sand and earth

Soil, sand or earth from countries other than the United States (except Hawaii) may not be imported, except for scientific purposes, and accompanied by a permit.

Sand, soil or earth as ships' ballast from countries other than the United States (except Hawaii) may not be imported, unless a statement is presented to the port warden declaring that the ballast consists of sand or gravel from a river bed or shore line within tidal waters or a fresh water rice bed, open working sand or gravel pit, virgin desert sand, colliery refuse or building or quarry rubble.

FEDERAL REPUBLIC OF GERMANY

Ordinance of 28 July 1960 amends the Plant Protection Ordinance of 23 August 1957 (see *FAO Plant Prot. Bull.* 6 : 27-29. 1957). The amendment contains the following provisions.

The enforcement of the requirement regarding obligatory certification of cereals, oil cakes and dry pulses (see *FAO Plant Prot. Bull.* 7 : 71. 1959) is to be postponed to 1 March 1965. Provisions regarding inspection and treatment, however, remain unchanged.

The following plants, grown in Bulgaria and Yugoslavia, except their fruits and seeds, are added to the list of plants, the introduction of which is prohibited : *Prunus armenica, P. triloba, P. insititia, P. cerasifera, P. persica and P. domestica.* This measure is to prevent the introduction of plum pox of stone fruit.

The list of points of entry has been amended and supplemented.

UNITED ARAB REPUBLIC (Syrian Region)

Decree-Act No. 237 of 17 July 1960, published in the *Journal officiel* No. 160 on 18 July 1960, regulates the import and export of plants and plant products and establishes powers of the Minister of Agriculture in this connection. It repeals Decree No. 132 of 7 October 1953 and all other dispositions that may be contrary to the present Decree.

The present Decree defines "plants" as any plants or any parts thereof (whether dead, living, or dried), and "plant products" as any products of plant origin including those which have been processed without affecting their plant nature.

The import and export of plants and plant products is prohibited, unless they have been inspected by the Phytopathological Service and found to be healthy and to conform with requirements of the province or of the importing country.

The entry of plants and plant products infected or infested with diseases or pests is prohibited, except where disinfection or disinfestation is possible. Exemptions may be granted from this requirement if the diseases or pests involved also occur in the province and if the imports will not menace its agriculture.

The Minister of Agriculture is empowered to issue decrees to determine the plants whose import is to be prohibited or authorized, to determine the pests and diseases whose entry is to be prohibited or may be permitted after treatment, and to establish the points of entry and conditions governing the import, export and transit of plants and plant products.

NEWS AND NOTES

BOLIVIA ADHERES TO INTERNATIONAL PLANT PROTECTION CONVENTION

The Government of Bolivia became party to the International Plant Protection Convention on 27 October 1960, when its instrument of adherence was received by the Director-General of FAO. The number of governments contracting to the Convention, including both signatory and adhering members, is thus at the time of writing 42, namely: Argentina, Australia, Austria, Belgium, Bolivia, Cambodia, Canada, Ceylon, Chile, Denmark, Dominican Republic, Ecuador, Egypt, El Salvador, Federal Republic of Germany, Finland, France, Greece, Guatemala, Hungary, India, Iraq, Ireland, Israel, Italy, Japan, Korea, Laos, Luxembourg, the Netherlands, New Zealand, Nicaragua, Norway, Pakistan, Republic of the Philippines, Portugal, Spain, Sweden, the Union of South Africa, the Union of Soviet Socialist Republics, the United Kingdom and Yugoslavia.

A NEW INTERNATIONAL JOURNAL *Phytophathologia Mediterranea*

Phytopathologia Mediterranea is a new journal devoted to plant pathological problems in the Mediterranean area. As indicated in the introduction in its first issue, the Mediterranean area is regarded not only as a historical unity, but possesses peculiar climatic, ecological and agricultural features which determine the characteristics of its plant disease problems. The publication of this new journal, it is hoped, will contribute to the revival of personal connections among the Mediterranean students of phytopathology and also to a more complete and thorough co-ordination of plant disease studies in the area.

The first issue of this journal appeared in August 1960, containing original papers in various languages. Summaries in English and French are provided. Each volume consists of four issues.

The journal is published under the direction of Prof. G. Goidanich, Istituto di Patologia Vegetale dell'Università, Via Filippo Re 8, Bologna, Italy, and Prof. A. Ciccarone, Istituto di Patologia Vegetale dell'Università, Via Amendola 165/A, Bari, Italy, to whom contributions may be sent and from whom further information may be obtained.

Stabilimento tipografico FAUSTO FAILLI - Via Tuscolana, 128, Roma

GRASS COVER OF AFRICA

FAO Agricultural Studies No. 49

This important work is actually a summary in map form of the extent of present knowledge of the grass cover of Africa which can be used as a guide not only in the field of range or grassland management, but also in the wider reaches of land use generally. Included with 143 pages of text is a six-color 1 : 10,000,000 map in separate folder of Africa's grass cover as compiled from discussions and correspondence with a great number of specialists in Africa and elsewhere.

Textually, the study deals with ecological relationships of Africa's grass cover and types of vegetation with which such cover is associated. Twenty-three genera used to distinguish the various associations are also discussed, as well as types of the grass cover itself. There are a number of appendixes, including an index of African grass cover types and their geographical distribution.

Publication of this study has resulted from a decision by FAO to map the grass cover of Africa first, and then to use the experience and technique so gained to prepare maps of other parts of the world. It represents a first step forward toward the long-term objective of mapping the grazing resources of the world's underdeveloped countries on some basis of value to those concerned with the improvement of grassland management and the livestock industries dependent on them.

Available at FAO, Rome, Italy, or from local sales agents in English, French and Spanish editions.

$2.50 or 12s. 6d.

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